Docket No. 56778 (70820)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT:	Y. Sasaki et al.		
U.S.S.N.:	10/019,743	GROUP:	1653
FILED:	December 28, 2001	EXAMINER:	A. U. Desai

FOR: PROCESS FOR PREPARING LH-RH DERIVATIVES

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 CFR 1.131

The undersigned declare as follows:

1. We are co-inventors of the above-identified patent application assigned to the Takeda Chemical Industries, Ltd.

2. Prior to December 18, 1998, we made and successfully isolated Purified leuprorelin or a salt thereof having a sum of all impurities of less than about 1% as disclosed and claimed in the above-identified patent application.

3. That the purified leuprorelin we made and isolated prior to December 18, 1998 also included purified compositions in which the content of 5-oxo-Pro-D-His-Trp-Ser-Tyr-D-Leu-Leu-Arg-Pro-NH-CH_2-CH_3 or a salt thereof was about 0.3% or less.

4. That the purified leuprorelin we made and isolated prior to December 18, 1998 also included purified leoprorelin compositions in which the impurities were racemic isomers of the LH-RH derivatives and/or highly polar related substances.

5. Attached as Exhibit 1 is a true and accurate copy of laboratory notebook records, as well as partial English translations of the notebook records, with dates deleted. The notebook records demonstrate that the purified leuprorelins as described in paragraphs 2, 3, and 4 above were made prior to December 18, 1998. Exhibit 1 shows, among other things, methods of characterization of the purified leuprorelin compositions, confirmation of reproducibility by scale-up, and analytical analysis for the purified leuprorelin as described in paragraphs 2, 3, and 4 above. On that Exhibit 1, the references to "S3-TAP and TAP-144" designate the particular purified leuprorelin compositions having less than 1 % impurities.

5. We hereby further declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both (18 U.S.C. 1001), and that such willful false statements may jeopardize the validity of the above-identified application or any patent issued thereon.

Date: December 17, 2004 Y. Sasaki
Yasuhiro Sasaki

Date: December 17 2004 K. Shimizu
Katsuji Shimizu

Exhibit A

メタンスルホン酸 ×1.25
反応温度 (15°C) による HIs. 抑制 効果確認と調整

1. 300ml 4頸フラスコ	1. 回収 513.39 g
2. メタンスルホン酸 178+~~[illegible]~~ 323 (1.25)	2. 混用
3. エタノール 13.4g	3.
4. 冷却 g	4. 8:42 → (11.95g)
5. HBS-TAP 投入	5. 12.87 g → (TAP 11.0g 相当分)
6. 〃 一括添加 (20°C以下)	6. 8:55 (7.0) ~ 9:07 (8.0)
7. 反応 g (3Hr)	7. 9:07' (9.0) (Temp セット 外 8.9 内 10.3~10.5) ボール状顆粒 [illegible] 急冷 Temp
	10:08' 溶けた溶解
8. 終了	8. 12:08 (10.5°C)
	(オ) (751.63 / 751.62) (238.24 / 237.63) f=0.9974 → 濃縮
9. 反応液を-5°C以下に冷却した希釈酢酸エチル溶液 酢エチ 46ml に注ぎ込む	9. 12:08 (-10) ~ 12:32 (-5°C) (氷浴不用とはった)
10. 30' 攪拌 10' 静置 アルカリ層は酢エチ相で抽出	10. 12:32 (7.0) ~ 13:02 (5.0) ~ 13:12 (6.0) 水層 767.04 g
11. アルカリ物 溶解 重曹水 添加	11. 13:16 (6.0) ~ 13:20 (6.0)
12. pH 調整 酢エチ 89ml 添加	12. 5.01 (6.0) → 4.49 (5.0) (酢酸 8.5ml)
13. 10' 攪拌 30' 静置 水層 採取	13. 13:25 (6.0) ~ 13:35 (5.0) ~ 14:05 (5.0) 水層 652.76 g
14. 水層を pH 2.4 にて洗い込み 酢酸エチル 89ml 添加 10' 攪拌 30' 静置 水層を採取	14. 14:13 (7.0) ~ 14:23 (3.0) ~ 14:53 (5.0)
	(オ) (679.38 / 678.26 f=0.9984) f=0.9958 → 濃縮後
15. 外温 25°C にて 濃縮	15. 15:10 (25) ~ 15:40 (25)
	(615.16 / 613.96 f=0.9980)
	水分 0.00768 / H_2O
	0.07%

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○ HBS-TAP (粉) … 現場品 Lot 076 (P: 92.87%)

○ 添加 0.75%食塩水 溶解 (30.76g)
○ 緩衝液 pH 8.21 → 4.08 (酢酸 11.5 ml)

残 HBS-TAP
0.0177%   0.0422g/11.95 = 0.35%

収率 4.23%   10.085/11.0 = 91.68%
反応後 91.68%
濃縮前 18.72%
〃後 85.34%
一夜後 87.61%   11/30 1.58% 88.73%

類縁物質 (USP法)

	D-Trp	D-Ser	D-His	L-Leu
反応後	1.60	0.08	0.23	0.90
濃縮前	—	—	0.25	0.76
〃後	—	—	0.26	0.92
一夜後	—	—	0.24	0.93 ✓
保存	=	(0.01)	0.25	0.94

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スケールUPによる再現性の確認 (1)

従来法の4工程 (HP-20 → CM-23 → HP-20 → LH-20) から1工程削減 (HP-2HG → HP-20SS → LH-20) を目的にスケールUPにて新法を確認する。

HP-2HGによるクロマト精製 C-TAP → SI-TAP
詳細データシート参照、

結果	D-Trp体	D-Ser体	D-His体	L-Leu体	面百%	回収率
C-TAP	0.01%	0.01%	0.26%	0.96%	98.78/TMAB	88.73%
SI-TAP	0.12%	0.03%	0.20%	0.68%	9888/9780	86.45%

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Read and understood by:	Date (mm/dd/yy) / /	Read and understood by:	Date (mm/dd/yy) / /

TAP-144(500 工程)カラムクロマト実験記録

HP-2MG 5ºC直液(ジャケット)

EXP No. 10279-88 樹脂量 400 ml

Sheet 1

Operation Date	Time	Charge Name	Charge pH	Charge volume (ml)	Charge weight (g)	Fraction Fr. No.	Fraction pH	Fraction volume (ml)	Fraction weight (g)	社内法 TAP-144 conc. (w/v%)	Net (g)	yield (%)	類縁物質(%)[TAP-144との面積比] 高極性物質	L-Leu⁴	[illegible]	USP法 類縁物質(%)[TAP-144との面積比] D-Trp³	D-Ser¹	D-His²	L-Leu⁴	備考
9/10		C-TAP 1258-18	4.41		400.0					1.576	6.31		5.09	0.93	98.98	0.01	0.01	0.26	0.96	
10/10		PW 20ml で洗浄																		
		溶出液				1	5.62	415												
10:16		0.2N AcONa	6.2	800																
		溶出液				1	1.80	810												
15:10		0.05N AcONH4		1000																
		溶出液				1	6.10	1010												
8/10		10% EtOH		2000																
		溶出液				1	5.90	2030		0.00011	0.0023	0.04								
	16:30	0.05N AcOH				1	3.54	400												
9/20						2	6.13	102		0.00086	0.0009	0.054	7.41	=	89.76					
						3	5.04	100		0.00254	0.0025	0.094	24.10	=	94.57					
						4	4.09	100	99.07	0.172	0.170	2.80	4.96	=	98.27					
						5	4.39	140	136.03	0.8942	1.216	22.08	1.13	—	98.57					
						6	4.30	160	101.12	0.8825	0.892	16.22	0.71	0.12	98.83					
						7	3.63	1020	1023.66	0.2737	2.802	80.62	1.03	0.48	98.27					
						8	3.30	260	260.82	0.0657	0.1710	83.74	2.46	1.26	95.47					
						9	3.25	200	202.54	0.0525	0.106	85.03	4.22	1.32	94.45					
						10	3.23	200	208.98	0.042	0.088	86.42	5.04	1.52	92.84					
						11	3.17	200	258.80	0.033	0.097	87.95	6.16	1.63	92.07	0.15	—	0.26	3.64	
	16:30	35% EtOH 0.05N AcOH 混液 1000						10060		0.0842	0.842	101.29								
						主流液 濃縮後カラムにて	3.66		2009.32	0.2725	5.495	86.3	1.43	0.41	97.81	0.12	0.03	0.20	0.72	97.12
									40.16											
									1969.16		5.386	濃縮								
	10:07	外温 60℃ で濃縮																		
						SI-TAP 試験用にサンプリング	3.93		279.12	1.933	5.396	100.17	1.27	0.39		0.12	0.03	0.20	0.48	97.80
									18.22											
									260.80		5.04g		HP-20SSへ	→	10279-90へ					

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HP-20SSによるSt-TAPの精製

St-TAP 10279-88

HP-20SSの通液量は 15°C 樹脂量 2容積の175mL

詳細テクニート参照、

結果	D-Trp体	D-Ser体	D-His体	L-Leu体	面百%	回収率
St-TAP	0.13	0.04	0.20	不検出	99.54/99.46	90.23

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Read and understood by:	Date (mm/dd/yy) / /	Read and understood by:	Date (mm/dd/yy) / /

TAP-144(500 工程)カラムクロマト実験記録

HP-20SS 温度 15℃

EXP No. 10279-90 樹脂量 175 ml ＊樹脂量 2割増

Sheet 1 Bl-TAP lot 10279-88

Operation Date	Operation Time	Name	Charge pH	Charge volume(ml)	Charge weight(g)	Fraction Fr. No.	Fraction pH	Fraction volume(ml)	Fraction weight(g)	社内法 TAP-144 conc.(w/v%)	社内法 TAP-144 Net(g)	社内法 TAP-144 yield(%)	社内法 類縁物質(%) [TAP-144との面積比] 高極性物質	社内法 類縁物質(%) L-Leu	社内法 純度	USP法 類縁物質(%) [TAP-144との面積比] D-Trp	USP法 D-Ser	USP法 D-His	USP法 L-Leu	備考
	9:15	SITAP	3.90		560.01					1.883	4.90	100	1.31	0.13		0.13	0.04	0.20	0.15	
		PW洗浄		20ml																
	11:30	脱塩水																		
	11:30	0.1M AcONa	6.34	525ml		1	3.23	280												
	14:45	0.01M AcONH4		805		1	4.58	530												
	9:25	PW洗浄		175		1	6.04	810		2.1×10-5	1.7×10-4									
	10:15	脱塩水																		
	10:45	20%EtOH		200		1	6.35	175		8.2×10-5	6.5×10-5									
		脱塩水																		
	15:00					1	4.08	325		1.39×10-5	4.7×10-4									
	15:25					2	4.41	100		1.52×10-5	1.4×10-5									
	15:35					3	4.15	50												
	16:20					4	4.31	50		3.44×10-5	1.72×10-5									
	16:50					5	4.48	50		9.66×10-5	4.83×10-5	0.006								
	17:15					6	4.53	50	47.53	1.46×10-3	0.01	0.02								
						7	5.05	50	47.85	0.02	0.026	0.23	9.53							
						8	6.21	30	22.56	0.13	0.064	0.96	1.83							
						9	6.55	30	28.00	0.228	0.064	2.26	1.94			0.59	0.17	0.18	—	
	9:05	35%EtOH				10	6.82	16.5	15.40	4.392	0.065	3.18	1.20			0.53	0.13	0.19	—	
	10:05															0.24	0.07	×	—	
	18:00				主流	1	7.83	325	382.42	1.048	3.885	79.31	0.26		99.04	0.14	0.03	0.19		
	18:55				主流	2	8.89	120	114.56	0.424	0.485	89.21	0.37		99.04	0.09	0.04	0.21		
					主流	3	8.59	80	23.06	0.066	0.304	91.22	0.42		99.13	0.10	0.06	0.25→0.21		
					主流	4	8.27	25	22.17	0.485	0.107	92.41	0.34		99.16	0.10	0.07	0.23→0.25		
						5	8.13	20	19.39	0.488	0.095	95.34	0.50		99.42	0.09	0.09	0.33		
						6	7.14	20	17.06	0.425	0.074	96.85	2.85		98.97	0.12	0.39	0.48		
						7	6.25	21	18.82	0.262	0.049	97.86	6.57		97.24	0.10	0.57	0.72		
		Bl-TAP主流液 35%EtOH Fr-1~Fr-4					主流液		533.95	0.1815	4.384	88.86	0.47		99.53	0.13	0.04	0.20		DS換算 99.45
		固形分 90.23																		
		収 542.91 4.429					SITAP		30.01	14.145	4.24	86.63	0.46		99.54	0.13	0.04	0.20		99.46
						カラム洗浄			22.58		3.90									
												F5279-96A	HC30							

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LH-20による精製

S2-TAP 1029-90

通液条件は現行法

詳細データシート参照、

結果

	D-TAP3体	D-Ser4体	D-HIS体	L-Leu6体	面百%	回収率
S3-TAP	0.13	0.04	0.20	N.D	99.54/99.46	90.23
S4-TAP	0.13	0.04	0.20	N.D	99.54/99.42	97.21
製品	0.14	0.05	0.19	N.D	99.52/99.45	

水分測定

<SAMPLE NO.> 01-65
LOT NO. 008
FACTOR
BUR1 2.041 MG/ML
SIZE
B.G. 0.0 MICR 0
WT1 15.92153 G
WT2 15.63017 G
RESULT 3.432 %
BUR1 3.520 ML

SAMPLE NO. 144- 1

DATE [redacted] 10:55
SAMPLE NO. 144- 1
H2O 6287.7 MG
BLANK 0 MG
S.SIZE 0 G
0 G
0 G
H2O % 6287.7 MG
S.SIZE 2.7254 G
2.4865 G
0.2386 G
DATE 95/12/25 10:55
SAMPLE NO. 144- 1
H2O 6287.7 MG
BLANK 0 MG
S.SIZE 2.54 G
2.4865 G
0.2386 G
H2O 2.634 %

TAP-144(500 工程)カラムクロマト実験記録

LH-20 室温

EXP No. 1027996　樹脂量 145 ml

Sheet 1

Operation		Charge				Fraction				社内法						USP法				
										TAP-144			類縁物質(%)[TAP-144との面積比]			類縁物質(%)[TAP-144との面積比]				
Date	Time	Name	pH	volume(ml)	weight(g)	Fr. No.	pH	volume(ml)	weight(g)	conc. (w/w%)	Net(g)	yield(%)	高極性物質	L-Leu[1]	保持可能	D-Trp[2]	D-Ser[4]	D-His[3]	L-Leu[6]	備考
	10:20	SJTAP	2.70		27.42					14.44	3.96		0.45			0.13	0.04	0.19	—	
		研究用				1	6.21	25		0.0018	0.00041	0.01	6.07	4.827						
	12:00	0.075N AcOH	5.56	435																
	14:00																			
						1	5.85	30	29.86	0.000051	2.4-05	0.011	140.91							
						2	5.75	15	13.91	0.0000057	9.4-07	0.011	754.11							
						3	—	5.5	5.08	0.0000041	2.3-06	0.011	208.80							
	9:20					4	5.65	10	9.52	0.0000058	6.5-06	0.011	38.70							
	10:05					5	5.63	10	9.45	—	—	0.011	—							
	10:45					6	5.30	10	9.36	—	—	0.011	—							
	11:25					7	6.50	11	10.44	2.460	0.257	6.49	0.53			0.03	0.03	0.06	—	
						8	7.21	60	58.93	5.310	3.131	85.64	0.32	0.327		0.13	0.05	0.21	—	
						9	6.90	25	23.91	1.610	0.400	95.64	0.58			0.20	0.03	0.25	—	
						10	6.70	15	14.09	0.771	0.112	98.44	0.24			0.20	0.03	0.23	—	USP適否X
		SJTAP (1) Fr-8,9 Y=87.15% wt 80.58					7.24		80.09	4.17	3.277	83.27	0.46		98.54	0.14	0.04	0.21	—	99.39%
		SJ (2) Fr-7,8,9,10 Y=98.05% wt 101.8					7.13		101.76	3.573	3.635	91.50	0.60		98.41	0.13	0.04	0.20	—	98.38%
											(3.550)	(97.21)								
			製品	0.079g(水)	...	ロス有り)														
		SJTAP 濃縮液																		
							6.99		330	10.18	0.360	84.84	0.48		98.53	0.13	0.04	0.20		99.42
	10:00	凍結乾燥		99.63																
	~			−66.63																
	9:00			33.00				水分 TP法 3.43												
				−0.45g 残				USP法 2.64												
				32.55g																

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Partial English Translation of Laboratory Notebook Records

Page 18 (12258-18)

Methane sulfonic acid x 1.25
Confirmation of inhibition of His effect and preparation ~~at reaction temp. 15°C~~

1. 300 ml 4-neck flask	1. tare 513.39 g
2. methane sulfonic acid 17.8+ 32.3 (1.25)	2. including washing
3. phenol 13.4 g	3.
4. cooling S	4. 8:42
5. NBS-TAP weighing	5. 12.87 g (corr . to TAP 11.0 g)
6. ditto charge (8°C or lower)	6. 8:55 (7.0) - 9:07 (8.0)
7. reaction S (3 Hr)	7. 9:07 (9.0)
8. finished	8. 12:08
.....	.....
15. concentrated at 25°C of outer temp.	15. 15:10 (25)-15:40 (25)
	615.16
	613.96 f=0.9980
	aqueous layer loss
	0.0076g/H_2O
	0.07%

Takeda Chemical Industries, Ltd.

・MBS・TAP (dry) plant product Lot076 (P: 92.87%)
・potassium carbonate 0.25 min. additional dissolution (30.76 g)
・buffer pH 8.21 → 4.08 (acetic acid 11.5 ml)

remainder MBS・ TAP
0.0177% 0.0422 g/11.95 = 0.35%

reaction rate 4.23% 10.085/11.0 = 91.68%

reaction mixture	91.68%		
before concentration	78.72%		
after concentration	85.34%		
after overnight	87.61%	/.58%	88.73%

related substances (USP method)

	D-Trp	D-Ser	D-His	L-Leu
reaction mixture	1.60	0.08	0.23	0.90
before conc.	-	-	0.25	0.76
after conc.	-	-	0.26	0.92
after overnight	-	-	0.24	0.93
refrigeration		0.01	0.25	0.94

Confirmation of reproducibility by scale-up (1)

For the purpose of reducing one column (HP-2MG→HP-20SS→LH-20) from the previous 4 columns (HP-20→ CH-23→HP-20→LH-20), the new process is confirmed by scale-up.

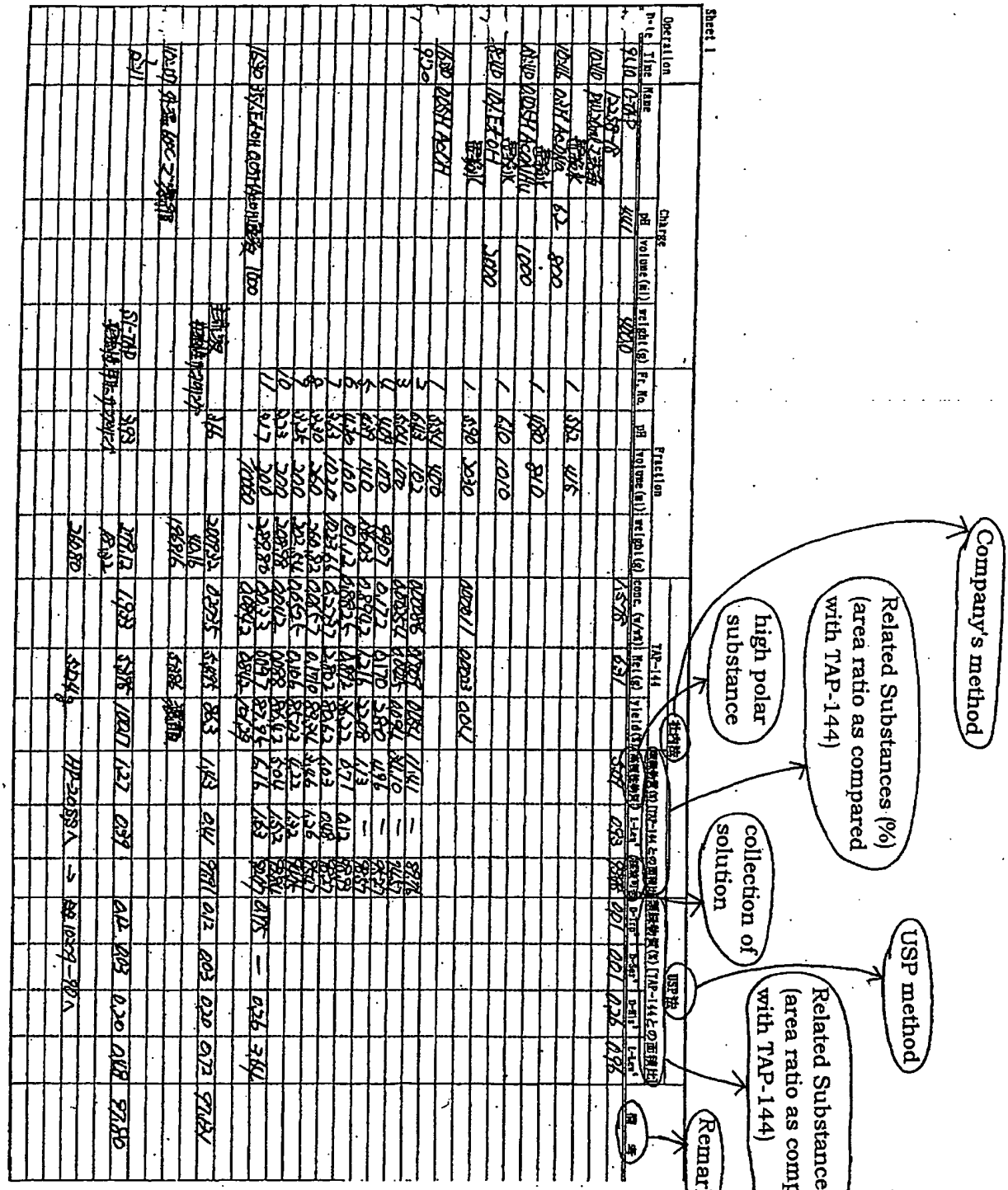
chromatographic purification by HP-2MG C-TAP→S1-TAP
cf. detailed data sheet

Results

	D-Trp^3 form	D-Ser^4 form	D-His^2 form	L-Leu^6 form
C-TAP	0.01%	0.01%	0.26%	0.96%
S1-TAP	0.12%	0.03%	0.20%	0.48%

	area %	recovery
C-TAP	93.98/77.43	88.73%
S1-TAP	98.08/97.80	86.45%

AP-144 (500 Step) Column Chromatography Experimental Record
HP-2MG 5° C passage (jacket)
EXP No. 10279-88 resin amount 400 ml

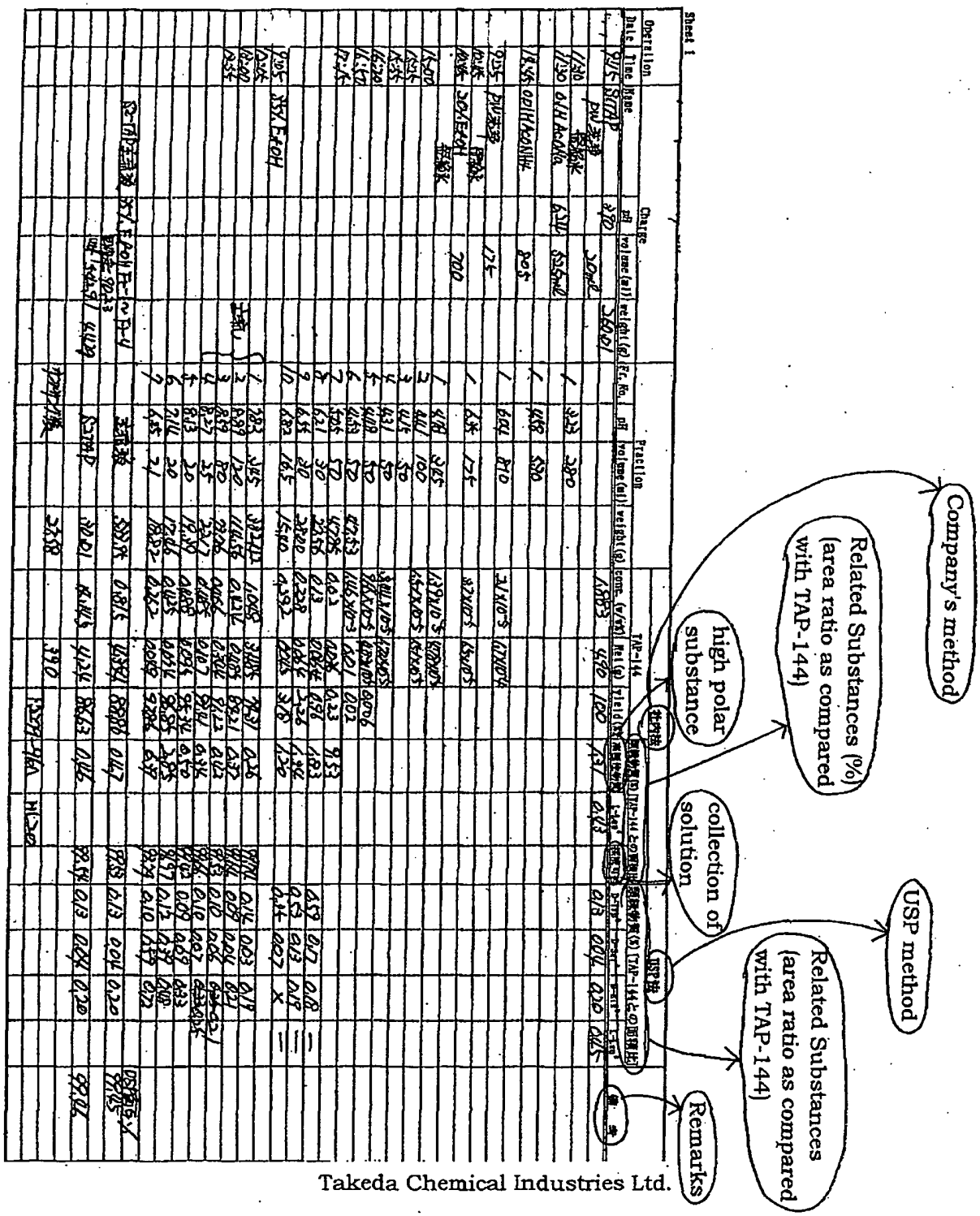


Purification of S1-TAP by HP-20SS
S1-TAP 10279-88
HP-20SS passage temperature 15°C, resin amount 20% up 175 ml
cf. detailed data sheet

Results

	D-Trp[3] form	D-Ser[4] form	D-His[2] form	L-Leu form
S2-TAP	0.13	0.04	0.20	not detected
	area %	recovery		
S2-TAP	99.54/99.45	90.23		

AP-144 (500 Step) Column Chromatography Experimental Record
HP-20SS Temp. 15°C
EXP No. 10279-90 resin amount 175 ml *resin amount 20% up
S1-TAP Lot 10279-88



Purification by LH-20

S2-TAP 10279-90

Passage conditions is the current method.

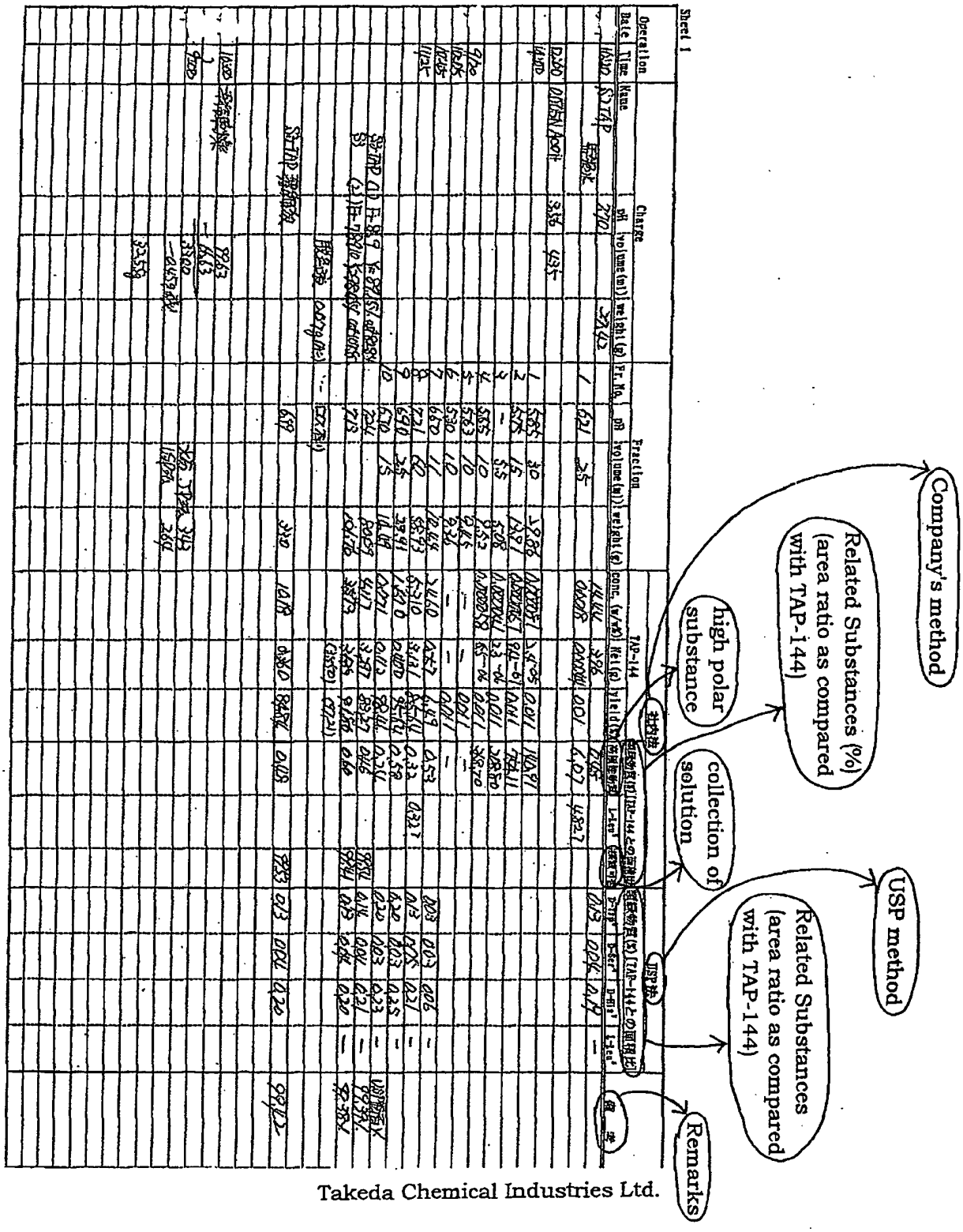
cf. detailed data sheet

Results

	D-Trp^{3} form	D-Ser^{4} form	D-His form	L-Leu^{6} form
S3-TAP	0.13	0.04	0.20	N·D
S4-TAP	0.13	0.04	0.20	N·D
product	0.14	0.05	0.19	N·D
	area %	recovery		
S3-TAP	99.54/99.46	90.23		
S4-TAP	99.54/99.42	97.21		
product	99.52/99.45			

Moisture Measurement

AP-144 (500 Step) Column Chromatography Experimental Record
LH-20 room temp.
EXP No. 10279-96 resin amount 145 ml



Summary of Records

Re: Details of obtaining a solution containing TAP-144 whose impurities content is 1% or lower and D-His^2 form content is 0.3% or lower

D-His^2 form content has been already reduced to 0.3% or lower by studying de-MBS reaction conditions before purification (C-TAP), and then, impurities in TAP-144 is reduced to 1.0% or lower by chromatographic purification.

Date	Steps	Experimental method	Results	Notebook No.
	MBSTAP ↓ C-TAB (corr. to Example 1)	Purpose: To confirm effect on control of D-His^2 form. Experimental method Amount of MESE charge: 1.25-fold Reaction time: 3 hrs Reaction temp.: 11°C	USP method area % D-His^2 form: 0.25%	12258-18 to 19
	C-TAP ↓ S1-TAP (corr. to Example 2)	Purpose: To purify C-TAP by HP2MG. Experimental method Passage temp.: 5°C (jacket) Resin amount: 400 ml C-TAP amount: 6.31 g Solvent: 0.05M AcOH	USP method area % S1-TAP D-His^2 form: 0.26%→ 0.20% TAP-144: 97.80%	10279-88 to 89
	S1-TAP ↓ S3-TAP (corr. to Example 3)	Purpose: To purify S1-TAP by HP-20SS Experimental method Passage temp.: 15°C (jacket) Resin amount: 175 ml C-TAP amount: 4.90g Solvent: 20% EtOH→ 35% EtOH	USP method area % S3-TAP D-His^2 form: 0.20%→ 0.20% TAP-144: 97.80%→ 99.46%	10279-90 to 91
	S3-TAP ↓ S4-TAP (corr. to Example 4)	Purpose: To purify S3-TAP by LH-20 Experimental method (current method) Passage temp.: room temp. Resin amount: 145 ml C-TAP amount: 3.96g Solvent: 0.005N AcOH	USP method area % S4-TAP D-His^2 form: 0.20%→ 0.20% TAP-144: 99.46%→ 99.42%	10279-96 to 97

Abbreviations in Notebook	Examples in the Present Application
MBSTAP	5-oxo-L-propyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-D-leucyl-L-leucyl-Nw-p-methoxybenzenesulfonyl-L-arginyl-N-ethyl-L-prolinamide
C-TAP	Aqueous solution (1) of Leuprolide
S1-TAP	Aqueous solution (2) of Leuprolide
S3-TAP	Aqueous solution (3) of Leuprolide
S4-TAP	Concentrated fractions containing leuprolide eluted with a 0.005 M aqueous solution of acetic acid